Assessment of Paracellular Permeability in *In Vitro* Barrier Models

For Research Use Only - Not Human or Therapeutic Use

INTRODUCTION

Maintaining selective permeability is a fundamental requirement for the physiological function of biological barriers, including the intestinal epithelium, the blood-brain barrier (BBB), and the vascular endothelium. These barriers protect internal environments from harmful substances while facilitating the transport of nutrients and signaling molecules. Central to this selectivity are the tight junctions (TJs), which form a continuous intercellular seal that regulates the paracellular pathway—the space between adjacent cells.

1. STRUCTURAL DETERMINANTS OF BARRIER INTEGRITY

TJs are complex protein assemblies comprised of integral membrane proteins, such as occludin and claudins, and cytoplasmic accessory proteins like zonula occludens. Occludin, a 65 kDa transmembrane protein, is essential for maintaining TJ stability and is highly regulated by phosphorylation/dephosphorylation pathways. Claudins, another major TJ component, are critical determinants of pore size and charge selectivity of the paracellular barrier.

To evaluate the barrier functions and conditions using culture cells, the Transwell chamber system is an indispensable tool in permeability research, providing a robust *in vitro* platform to simulate complex biological barriers. Its utility stems from its unique dual-compartment architecture, which allows for the creation of polarized cell models that closely mimic physiological conditions.

The primary advantage of the Transwell system is its ability to facilitate the growth of polarized cell monolayers. Cells seeded on the porous membrane of the insert differentiate to form distinct apical (upper) and basolateral (lower) surfaces. A critical feature of the Transwell system is the ability to conduct bidirectional transport assays.

The system provides a clear, quantitative measure of transport by determining the apparent permeability coefficient. Adding a substance like Fluorescein isothiocyanate-labeled dextrans (FITC-dextrans) to the donor chamber and collecting samples from the receiver chamber at defined time intervals provides an accurate calculation of the rate of paracellular transport across the barrier.

2. MEASURING PERMEABILITY: THE ROLE OF FLUORESCEIN LABELED-DEXTRAN

Fluorescein-labeled dextrans, such as FITC-dextrans, are widely used as paracellular markers because they are hydrophilic, non-toxic, and available in a range of molecular weights (e.g., 4 kDa, 7 kDa, 40 kDa, 70 kDa). Dextran permeability is directly linked to the physical dimensions of the TJ pores. The use of different molecular weight dextrans in studies can provide valuable information about TJ conditions. For example, low molecular weight dextrans (e.g., 4–7 kDa) are useful for detecting subtle changes in barrier integrity or the opening of small pores. Higher molecular weight dextrans (e.g., 70 kDa) are used to simulate the movement of larger macromolecules or to evaluate severe barrier breakdown.

Description	Amount	Catalog #
FITC–labeled Dextran - 4 kDa	25 mg/ml x 5 ml	4013
FITC–labeled Dextran - 40 kDa	25 mg/ml x 5 ml	4009
TRITC–labeled Dextran - 70 kDa	25 mg/ml x 5 ml	4014

For more dextran sizes or types, please visit the following webpage or contact support@chondrex.com.

3. MODEL-SPECIFIC CONSIDERATIONS

The following are typical cell models presenting unique characteristics that must be addressed during permeability testing:

3-1. Intestinal (e.g., Caco-2): These cells require 21 days to fully differentiate and polarize, developing a brush border and high TEER values that correlate well with human intestinal absorption (1–3).

3-2. Blood-Brain Barrier (e.g., hCMEC/D3): This immortalized human line mimics the highly selective BBB. It is particularly sensitive to pro-inflammatory cytokines like TNF-alpha, which can induce rapid occludin phosphorylation via MAPK pathways (p38 and ERK1/2), leading to enhanced permeability (4–6).

3-3. Vascular Endothelial (e.g., HUVEC): Often used to study inflammation and angiogenesis, HUVECs represent the peripheral vasculature. Their permeability is highly responsive to external stimuli, including shear stress from fluid flow and gradients of chemoattractants like CXCL-8 (7, 8).

4. SUGGESTED PROTOCOLS FOR FITC-DEXTRAN PERMEABILITY ASSAYS

4-1. Caco-2 cells

Caco-2 cells are seeded onto 24-well Transwell inserts (0.4 µm pore size) at 2 x 10^5 cells/cm^2 and cultured in DMEM containing 10% FBS and non-essential amino acids for 21 days post-seeding to achieve complete differentiation. For permeability evaluation, inserts are washed with HBSS, and 1 mg/ml FITC-dextran (4 or 40 kDa) is added to the apical chamber. Basolateral aliquots are sampled at 30, 60, 90, and 120 minutes. Fluorescence was quantified to compute the apparent permeability coefficient.

4-2. hCMEC/D3 cells

Transwell inserts (3.0 µm pore size) are pre-coated with 5 mg/ml Type I collagen prior to seeding hCMEC/D3 cells at 2.5 x 10^5 cells/cm^2. Cells are maintained in EBM-2 for 7 days until confluent. To induce barrier disruption, monolayers are stimulated with 10 ng/ml TNF-alpha for 24 hours. Permeability is assessed by replacing apical media with 1 mg/ml 4 kDa FITC-dextran in HBSS, followed by a 1 hour incubation at 37°C under gentle agitation. Tracer translocation to the basolateral compartment is quantified via a fluorescence microplate reader.

4-3. HUVEC cells

HUVECs are immobilized on a fibrin or collagen gel matrix within a microfluidic channel to simulate physiological shear stress conditions. A steady-state concentration gradient of 70 kDa FITC-dextran is generated utilizing a parallel-flow microfluidic architecture. Transendothelial diffusion into the extravascular matrix is tracked via real-time confocal microscopy. Permeability dynamics are mathematically correlated with morphological remodeling under inflammatory conditions.

5. CALCULATION AND EVALUATION

Individual calibration curves are created charting fluorescence measurements (FI values) against concentration. The FI values of the samples are then converted into the amount of FITC-dextran that has transferred across the monolayer. This amount is used to calculate the permeability of the FITC-dextran across the monolayer.

For more information or questions about calculations, please contact support@chondrex.com.

16928 Woodinville-Redmond Rd NE Suite B-101 Woodinville, WA 98072

Phone: 425.702.6365 or 888.246.6373 Fax: 425.882.3094

www.chondrex.com support@chondrex.com

REFERENCES

1. K. C. Fein, J. P. Gleeson, A. N. Newby, K. A. Whitehead, Intestinal permeation enhancers enable oral delivery of macromolecules up to 70 kDa in size. *Eur. J. Pharm. Biopharm.* **170**, 70–76 (2022).
2. S. Beutheu Youmba, L. Belmonte, L. Galas, N. Boukhettala, C. Bôle-Feysot, P. Déchelotte, M. Coëffier, Methotrexate modulates tight junctions through NF-κB, MEK, and JNK pathways. *J. Pediatr. Gastroenterol. Nutr.* **54**, 463–470 (2012).
3. A. Watari, M. Hashegawa, K. Yagi, M. Kondoh, Homoharringtonine increases intestinal epithelial permeability by modulating specific claudin isoforms in Caco-2 cell monolayers. *Eur. J. Pharm. Biopharm.* **89**, 232–238 (2015).
4. Y. Ni, T. Teng, R. Li, A. Simonyi, G. Y. Sun, J. C. Lee, TNFα alters occludin and cerebral endothelial permeability: Role of p38MAPK. *PLoS One* **12**, e0170346 (2017).
5. C. J. Czupalla, S. Liebner, K. Devraj, In vitro models of the blood-brain barrier. *Methods Mol. Biol.* **1135**, 415–437 (2014).
6. R. K. Sajja, S. Prasad, L. Cucullo, Impact of altered glycaemia on blood-brain barrier endothelium: an in vitro study using the hCMEC/D3 cell line. *Fluids Barriers CNS* **11**, 8 (2014).
7. E. Monaghan-Benson, E. S. Wittchen, In vitro analyses of endothelial cell permeability. *Methods Mol. Biol.* **763**, 281–290 (2011).
8. J. Pauty, R. Usuba, H. Takahashi, J. Suehiro, K. Fujisawa, K. Yano, T. Nishizawa, Y. T. Matsunaga, A vascular permeability assay using an in vitro human microvessel model mimicking the inflammatory condition. *Nanotheranostics* **1**, 103–113 (2017).

16928 Woodinville-Redmond Rd NE Suite B-101
Woodinville, WA 98072
Phone: 425.702.6365 or 888.246.6373
Fax: 425.882.3094
www.chondrex.com
support@chondrex.com